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Novel insights into biochemical and hormonal factors regulating floral transition in mango (*Mangifera indica* L.)

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Alternate or biennial bearing is an alternating pattern of large and small fruit yield occurring in consecutive years in mango, which is a major issue related to erratic productivity. This bearing behaviour is known to be regulated by internal physiological cues including biochemical and hormonal status of the adult shoots and accessibility to external stimulus that influences floral transition. This study aims to compile evidences to establish biochemical and hormonal patterning in floral transition utilizing standard protocols of colorimetric and transcriptomic studies. Floral tissues exhibited temporal pattern of higher accumulation of chlorophyll (0.40 to 0.99 mg gm^{-1} tissue in Chausa), total sugar (8.83 to 26.65 mg gm^{-1} tissue in Dashehari) and phenol content (0.21 to 0.83 mg gm^{-1} tissue in Dashehari), implying sufficient built-up of these in bud burst stage to be associated with floral morphogenesis. Furthermore higher accumulation of anthocyanins and auxin content with concomitant lower gibberellins in floral flush in all varieties validate the crosstalk among pathways to regulate the outcomes of floral transition. The genes sucrose synthase, sucrose phosphate synthase 1 (log 2 fold change value upto 4.98), ATP synthase, polyphenol oxidase and auxin response factor were found to be differentially expressed in the floral buds as observed by transcriptome data which corroborate the leads obtained by biochemical and hormonal assays. The study gives novel insights into the role of genes implicated in accumulation of carbohydrate reserves and establishment of hormonal gradients, that have decisive role in the flowering processes in mango.

Keywords: *Mangifera indica*, alternate bearing, floral transition, biochemical factors, hormonal factors

Introduction

Mango (*Mangifera indica* L.) is one of the most popular fruits of Indian origin, belonging to the family Anacardiaceae and has a long association with culture and religion in different parts of the country. Being indigenous to India, there exists enormous diversity and accordingly, more than 1000 varieties are known to exist in the country. Genetic studies have revealed *M. indica*, to be a diploid fruit species (2n = 2x = 40)[1], while reference of polyploidy has also been made[2]. It's out-crossing nature and perenniality has resulted in heterogeneity for many traits including flowering and fruiting, there by influencing the slow pace of breeding and understanding of complex traits. Genome heterozygosity and extensive evolution and domestication of the genome are ascribed to the uniqueness and diversity of mango qualities[3]. Flowering is one of the complex traits in mango, that has a direct relationship with fruiting and yield and many of its cultivars are alternate or shy bearers. Alternate bearing or biennial bearing is an alternating pattern of large and small crops occurring in many fruit species that is a major problem for commercial cultivation due to erratic productivity in different years. Regulation by internal physiological cues including the biochemical and hormonal status of the adult shoots and accessibility to external stimulus is implicated in the floral transition from a vegetative shoot[4]. Some of the key parameters associated with floral transition are internal factors: mobilization of carbohydrates, hormonal ratio, phenolic compounds, enzymes, and genetic factors that respond to changing temperature and photoperiod[5]. Furthermore, mango flowering phenology is unsynchronized due to erraticism of branches[6], or difference in the age of shoots and also the implication of long-distance signal due to the physical size of the tree, so that some shoots remain vegetative even in floral inductive conditions. Number, the timing of vegetative flushes, and crop load in the previous year are other important factors, critical for regularity in flowering[7].

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Flower bud differentiation or floral initiation in mango involves distinct changes in phytohormones and mobilization of carbohydrates from source to sink, that are dependent upon climatic conditions, shoot age and size, besides genetic characters. Experimental evidence indicates that maturity of terminal shoots and accumulation of carbohydrates in the shoot apex is in some way associated with the synthesis of the floral stimulus, the absence of which can result in a lack of flowering or biennial bearing in many mango cultivars[8]. Davenport[9] established that the maturity of terminal shoots and accumulation of carbohydrates in the shoot apex was linked to the synthesis of the floral stimulus in the shoot. Earlier researchers were also of the view that early initiation and cessation of growth followed by periodical quiescence or dormancy was critical for proper physiological maturity of apical bud. However, it has now been established that flower bud differentiation depends upon the 'on' and 'off' year phase of the tree rather than the initial cessation of growth of shoots, as observed by Kulkarni[10] and Reddy[11]. Besides carbohydrate reserves, other florigenic promoters such as phenols, chlorophyll, anthocyanin pigments and phytohormones such as auxin and gibberellin play important roles in the floral induction process. Relationships between flowering, fruiting in the previous year and carbohydrate accumulation in shoots are some of the decisive factors that have been associated with alternate flowering behaviour to a large extent[12].

The hormonal concept of flowering in mango implies that the cyclic synthesis of floral stimulus in the leaves and the difference between two such cycles would determine the flowering behaviour of a cultivar[13]. Some reports indicate that the isoprenoid pathway associated with gibberellin biosynthesis partially regulates the biosynthesis of other vital phytohormones such as ABA and cytokinins[14]. Other florigenic promoters such as phenols, chlorophyll, anthocyanin pigments and phytohormones are also known to play important role in the floral induction process. Patterning of auxins and gibberellins responsible for floral induction was proposed by Kachru *et al*[15-16]. Nonetheless, there exist deviations from this generalized phenology exhibited by individual trees, cultivars, seasons and tropical and subtropical environments and have therefore have been the focus of research for a long time. Even though mango flowering is an important subject of research, but it has mostly focussed on developing suitable agro-techniques to induce regular cropping or control biennial bearing. This study aims to compile substantiation to establish biochemical and hormonal patterning in floral transition in four important commercial cultivars that vary in their behaviour for flowering intensity and frequency. It also explores the molecular pathways for corroboration of findings evinced from the biochemical data by identifying differentially expressed genes responsible for the associated biochemical factors, endogenous hormones, and their transporters.

Materials and Methods

Four important commercial mango cultivars of the subtropical India were utilized for the study. The cultivars Dashehari, Langra, Chausa and Amrapali (Fig. 1) were selected due to their differential behaviour for flowering attributes particularly, flowering regularity and intensity in the subtropics, as per below:

Dashehari: Mid season, heavy floral induction, alternate bearing.

Langra: Mid-late bearing, heavy floral induction, strictly alternate bearing.

Chausa: Late season, shy floral induction, erratic bearer.

Amrapali: Late season, prolific floral induction, regular bearing.

Age of selected trees was approx. 20 - 25 years, healthy, with stabilized yield and maintained under uniform cultural practices. Two types of shoots were tagged: the mature shoots so called flowering bearing shoots yielded (i) Flower bud swelling stages (FBS) (BBCH scale 017) whereas, the new flush that continues to grow as vegetative flush yielded, (ii) Vegetative bud swelling stage (VBS) (BBCH scale 511). The fully mature middle leaves of the shoots were selected and sampled from these tagged shoots towards measurement of biochemical parameters and hormonal analysis. Thus, sampling was done from both types of the shoots in the month of October and January in regular bearing variety 'Amrapali' and alternate bearing cultivars 'Dashehari', 'Langra' and 'Chausa.

Estimation of Chlorophyll Content

The leaf chlorophyll content was estimated by Arnon's[17] method. In brief 500 mg of leaf sample was cut to small pieces and homogenized in a pre-cooled mortar

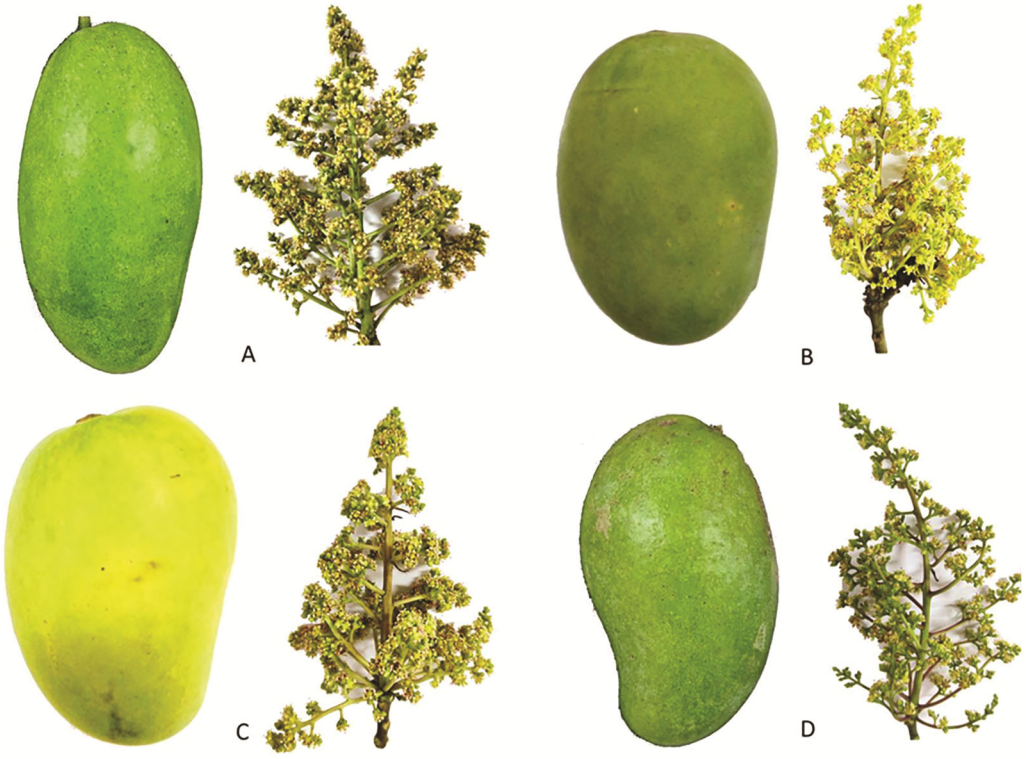

Fig. 1 — Mature fruit and full bloom panicle of mango cultivars; (A) Dashehari, (B) Langra, (C) Chausa, (D) Amrapali.

and pestle using 80% (v/v) acetone. A pinch of calcium carbonate was added while grinding. The extract was centrifuged at 3000 rpm for 15 min and made up to 25 ml with 80% (v/v) acetone. The clear solution was transferred to a colorimeter tube and the optical density was measured at 645 and 663 nm, against an 80% acetone blank in double beam spectrophotometer (UV 240). The content of chlorophyll 'a', chlorophyll 'b' and total chlorophyll were determined using the given equation

Chlorophyll 'a' (μ/g/ml) = (12.7 x O.D. at 663 nm) – (2.69 x O.D. at 645 nm)

Chlorophyll 'b' (μ/g/ml) = (22.9 x O.D. at 645 nm) - (4.68 x O.D. at 663 nm)

Total chlorophyll (μ/g/ml) = (20.2 x O.D. at 645 nm) + (8.02 x O.D. at 663 nm)

It was expressed as mg chlorophyll per gram fresh weight of the leaf.

Total Phenol Content Estimation

The total phenol content of the leaves was estimated by adopting the method of Bray and Thorpe[18] and the mean values were expressed as mg g^{-1} fresh weight. To 1 ml of alcoholic extract, 1 ml of folin-ciocalteau reagent (commercial folin-ciocalteau was diluted with distilled water in 1:2 ratio) and 2 ml of 20% sodium carbonate were added and shaken well. The mixture was heated in a boiling water-bath for 1 min and cooled under running tap water. The blue solution was diluted to 25 ml with distilled water and read at 650 nm in a spectrophotometer. Phenols were quantified using catechol as standard.

Total Soluble Sugars Estimation

For estimation of total soluble sugars the method of Yemm and Willis[19] was followed, 500 mg of dried leaf powder was homogenized in 10 ml of 80% methanol using mortar and pestle. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was saved and the pellet was re-extracted twice with the same volume of the solvent. The pooled supernatant was partitioned with an equal volume of petroleum ether in a separation funnel to remove the chlorophyll pigments. The methanolic layer was used for the determination of total soluble sugars. To 1 ml of the above extract, 4 ml of anthrone reagent was added from the sides of the test tube and boiled in a water bath for 10 min. The mixture was allowed to cool down to room temperature. A blank with 1 ml of distilled water in place of the sample was

used as reference. The optical density was measured at 625 nm. The soluble sugar content was calculated using glucose as the standard.

Anthrone Reagent Preparation

To 40 ml of distilled water, 100 ml of conc. H_2SO_4 was added. To 100 ml of the above mixture, 200 mg of anthrone was added and thoroughly mixed until a golden yellow colour appeared.

Anthocyanin Estimation

The anthocyanin content was estimated according to the method of Ranganna[20]. Five gram of leaf sample was cut in to small pieces and homogenized in a pre-cooled mortar and pestle using 50 ml of ethanol containing 1% HCL. Extract was filtered and stored in dark condition for 48 - 72 hrs. Optical density was measured at 535 nm, against methanolic HCL as blank in double beam spectrophotometer (UV 2100).

Auxin Estimation (Salkowski Method)

Five hundred mg of dried leaf powder was homogenized in 10 ml of 80% methanol using mortar and pestle. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was saved and the pellet was re-extracted twice with the same volume of the solvent. The methanolic layer was used for the determination of auxin. To one ml of the above extract, one drop of orthophosphoric acid and 2 ml of Salkowski's reagent was added. Pink colour develops, which confirms the presence of auxin. A blank with 1 ml of distilled water in place of the sample was used as reference. The optical density was measured at 530 nm. The auxin content was calculated using IAA as the standard.

Gibberellic Acid Estimation (Phosphomolybdic Acid Method)

Five hundred mg of dried leaf powder was homogenized in 10 ml of ethyl acetate with 0.05% Tween 20 using mortar and pestle. The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was saved and the pellet was re-extracted twice with the same volume of solvent. To 1 ml of the above extract, 15 ml of phosphomolybdic acid reagent was added from the sides of the test tube and boiled in a water bath for 1 hr. The mixture was allowed to cool down to room temperature and volume was made to 25 ml with distilled water. A blank with 1 ml of distilled water in place of the sample was used as reference. The optical density was measured at 780 nm. The gibberellic acid content was calculated using GA_3 as the standard.

Bioinformatic Analysis of Genes Related to Biochemical Factors

Analysis of the transcriptome data sets SRR11261956 and SRR11261955 were done to mine coding sequences (CDS) pertaining to key gene families associated with studied biochemical parameters which includes, chlorophyll, TSS, phenols anthocyanins and phytohormones and correlation with biochemical pathways. The coding sequences were retrieved and were subjected to BLAST analysis to reconfirm the function of the CDS. The redundant sequences were discarded and only those CDS matching with the specific gene families were used for further analysis. The functionally perfect coding sequences were translated into protein sequences and domains were predicted to confirm their structure and function. Protein sequences for CDS by translating coding sequences to amino acid/protein sequences (http://web.expasy.org/translate/). Multiple sequence alignment was performed in MUSCLE (www.ebi.ac.uk /Tools /msa /muscle/) as described by Edgar[21] and phylogenetic analysis was carried out as described by Dereeper *et al*[22] (www.phylogeny.fr/) and motif analysis was carried using, www.genome.jp/tools/motif/. Differentially expressed genes (DEGs) were determined on the basis of log2 fold change and p values, $Log2F \geq 2.0$ and $P \leq 0.01$ were used as cut-off values for filtering out the significance level of gene expression. Based on these cut-off values, the regulation of DEGs as significantly up or down regulated with respect to flower bud differentiation (FBD) was determined to validate findings of biochemical analysis.

Results and Discussion

The present study investigates and reveals the variations in biochemical parameters and hormonal states of 4 mango varieties of North India at flower bud swelling (FBS) stages and comparison with corresponding vegetative bud stages (VBS). The temporal accumulation pattern of the biochemicals and hormones was analysed along with corresponding molecular cues to implicate their contribution on the establishment of flowering potential of the adult shoots.

Variability in Chlorophyll Content Among Mango Varieties

Chlorophyll a, b and total chlorophyll content was found to depict an increasing trend from vegetative to floral transition in Dashehari, Langra and Chausa, with an exception in Amrapali (Fig. 2). The pigment

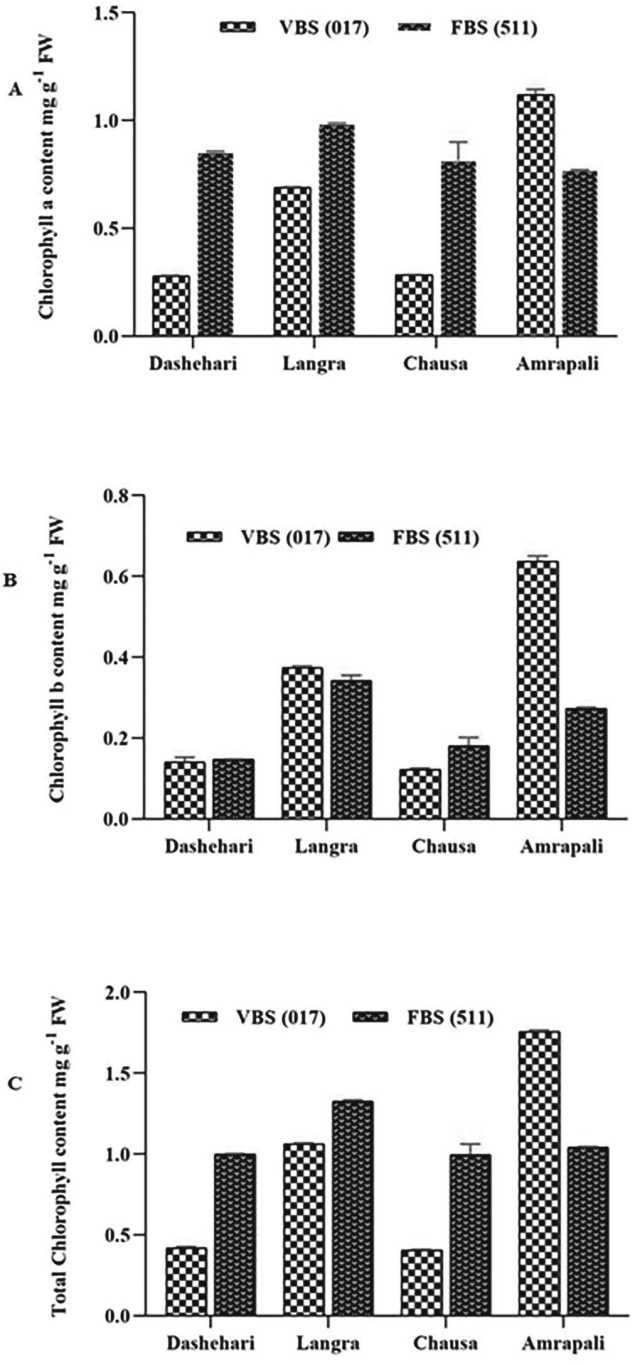

Fig. 2 — Variability in the chlorophyll content among mango cvs. (phenostages mentioned in brackets [47]).

content (chlorophyll a and b*)* was highest in Amrapali shoots harbouring vegetative buds (1.12 and 0.63 mg gm^{-1} tissue) and showed a slight decline in transition to floral shoot (0.77 and 0.27 mg gm^{-1} tissue). Same stages in alternate bearing varieties recorded a reverse trend i.e. the transition to floral shoot competency was marked with enhanced chlorophyll a and total chlorophyll (0.27 - 0.85 mg gm^{-1} and 0.41 - 0.99 mg gm^{-1} tissue in Dashehari) and chlorophyll b (0.140 - 0.146.84 mg gm^{-1} tissue in Dashehari), while in other two varieties (Fig. 1), chlorophyll b recorded declining trend in floral shoots. Even though, chlorophyll a is the main pigment for light harvesting and electron transport, minor variations in chlorophyll b during the floral transition are indicative of its role under stress. Earlier maximum chlorophyll content was reported in the period crucial for flower bud initiation in mango[23], that is corresponding to floral bud swelling stages. An interesting study on phytophagous insects attributed changes in leaf chlorophyll content to be associated with flowering[24]. Despite these advances, the nuclear genes involved in photosynthesis regulation remain poorly understood in trees due to their long-lived nature. Newer insights have revealed regulation of the chloroplast ATP synthase to be dependent upon the chloroplast thioredoxin system, the main redox regulation system in chloroplasts, which is directly coupled to the photosynthetic reaction[25].

Fluctuation of Sugar Content During Floral Induction

Estimations of total soluble sugar (TSS) indicated higher sugar content in floral bud-bearing shoots in all varieties. Significant variations for TSS were recorded in Amrapali flowering shoots (30.31 mg gm^{-1} tissue) and least in Dashehari vegetative shoots (8.83 mg gm^{-1} tissue) (Fig. 3A). Interestingly both Amrapali and Dashehari recorded higher enhancement in TSS content on floral transition (140.5 and 201.5%, respectively) in comparison to Langra and Chausa (38.7 and 79.9%, respectively). Hence, it can be confirmed that sugar content in shoots of different varieties exhibited a temporal pattern of carbohydrate reserve associated with flowering, sufficient built-up of which in the buds approaching bud burst stage ensured floral inductions under conducive conditions in mango. These results confirm with previous studies by Sen *et al*[26], where a higher accumulation of total carbohydrates, acid hydrolysable polysaccharide and protein content was reported during floral initiation. Corbesier *et al*[27] observed in model crop *Arabidopsis* that floral induction was associated with a large, transient and early increase in carbohydrate export from leaves, while in *Lolium temulentum* L. sucrose increased when flowering was induced[28]. It has been hypothesized that leaf non structural carbohydrate content is the driving force behind photosynthetic acclimation[29-30].

Accumulation of Phenols

Significant variation was recorded for the phenol content in all the varieties under study, wherein an increasing trend was recorded from vegetative differentiation to the floral bud formation stage. Among the varieties under study, Amrapali floral tissues had the highest phenol content (1.65 mg gm^{-1} tissue), while among alternate bearers Dashehari had comparatively more phenol accumulation than Langra and Chausa flowering shoots. Lower phenol content in vegetative bud swelling stages and higher in flowering shoots in all varieties implicates its accumulation to have a positive role in floral morphogenesis (Fig. 3B) and possible involvement of phenols in mango flowering. Higher phenols during flowering have previously been reported by Patil *et al*[31] in different mango cultivars as also by Misra and Dhillon[32]. The increase in phenols may not be of a direct effect on phenol biosynthesis but rather through its effects on phytohormone mediated increase in phenol content as stated by Rademacher[33].

Role of Anthocyanins

The quantitative difference of total anthocyanin content between floral and vegetative leaves was observed and significant variations were recorded. The flowering shoots of both Amrapali (3.10 mg gm^{-1} tissue) and Dashehari (2.91 mg gm^{-1} tissue) recorded higher anthocyanin content in floral bud swelling stages, followed by Langra and Chausa. A significantly lower level of anthocyanin content was found in all four cultivars in vegetative bud swelling stages (Fig. 3C). The role of high-concentration anthocyanins is hypothesized to act as a colored optical filter under normal light conditions and increasing the relative quantum of light in the green wave lengths inducing a photobiological effect leading to flowering. As this filtering effect is lost under low-intensity light, the hypothesis is supported by the report that cryptochrome, which is inactivated by green light, is involved in the shade-avoidance response[34]. Furthermore, stress-responsive flowering, leading to the accumulation of anthocyanins, suggests an involvement of the metabolic pathway regulated by phenylpropanoid pathway.

Role of Phytohormones (Auxins and Gibberellins)

Colorimetric estimations revealed significantly higher auxin content in floral flush as compared to vegetative shoots in all the studied varieties. Higher auxin concentration was recorded in Amrapali floral

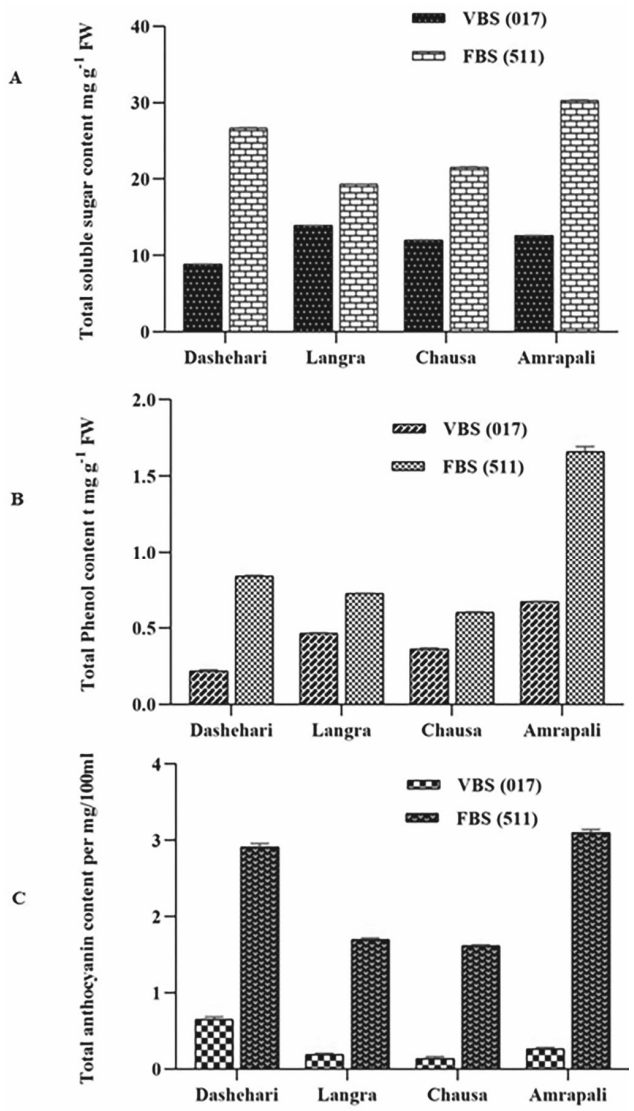

Fig. 3 — Role of sugar, phenol and anthocyanin in floral transition in mango cvs. (phenostages mentioned in brackets[47]).

flush (4.76 µg g^{-1} FW) and least in Chausa vegetative flush (0.88 µg g^{-1} FW) (Fig. 4A). Interestingly auxin content in Amrapali juvenile shoots (3.01 µg g^{-1} FW) was almost at par with the auxin content of floral flushes in alternate bearing varieties. This suggests that the role of auxin maxima was responsible for floral induction intensity as Amrapali is a regular bearer with most shoots participating in flowering, while other varieties with lower auxin in their vegetative shoots showed a typical alternating pattern of flowering with varied alternate bearing intensity[35]. At the flowering stage, there was a low rate of IAA oxidase activity which might have resulted in a greater amount of auxins in the leaves as also in other species[36]. It was also revealed that high-yielding

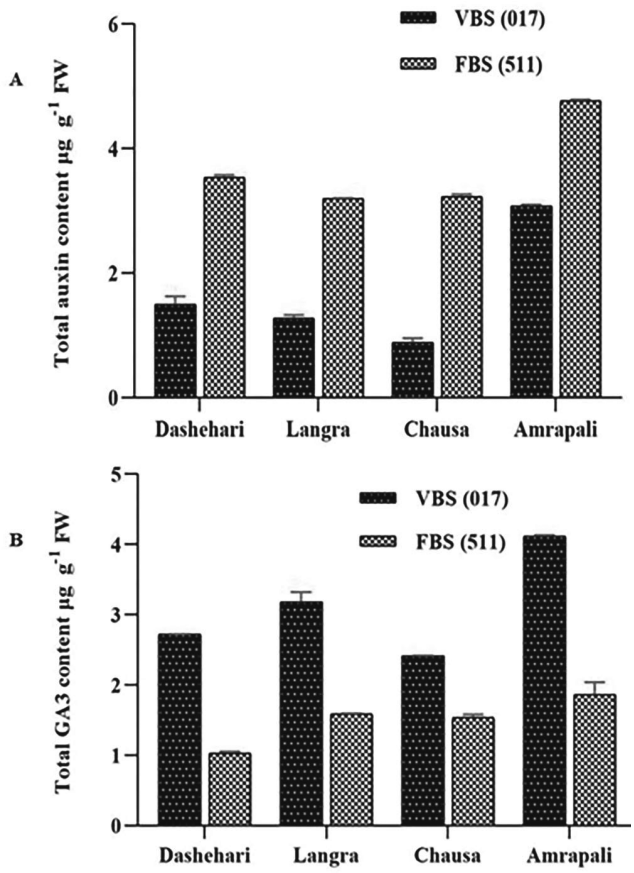

Fig. 4 — Endogenous hormones and their role for regulating floral induction in mango cvs. (phenostages mentioned in brackets[47]).

plants had favorable auxin balance through IAA oxidative degradation.

Additionally, a declining trend in gibberellic acid in floral flush as compared to the vegetative flush was observed in all the four mango cultivars (Fig. 4B), which explains the inhibitory potential of gibberellins on mango flowering[37]. These results are in agreement with the hormonal theory of flowering which states that higher auxin levels with a dip in temperature and decline in gibberellin levels promote inductive flowering in subtropical conditions in mango[38]. Furthermore, auxin : gibberellin ratio indicates that 3 fold enhancement is enough for floral transition in regular bearing variety Amrapali, whereas, higher fold expression (5 - 6 fold) is necessary for floral transition in Dashehari, Langra and Chausa. This was in corroboration with the findings of Upreti and Murti[39] and Singh[40], who confirmed that newly emerged leaves in the shoot of regular bearing cultivar such as Neelum were capable of synthesizing flower-inducing hormone. The activity of GA-like substances – greater in "off" year allowed postulation of the gibberellin inhibition in mango flowering[41-42]. The role of gibberellins in carbohydrate mobilization by stimulating their degradation to hexoses has earlier been proven[43]. A lower level of gibberellin-like substances and higher levels of cytokinin-like substances, growth inhibitors and ethylene have been indicated to be the prime factors favorable for induction of flowering in mango[44] .

Identification of DEGs Corroborating Biochemical Parameters

From the RNA Seq data (cv. Dashehari), a large number of genes associated with chlorophyll metabolism, phenol oxidation, sugar metabolism, anthocyanin metabolism, auxin and gibberellic acid regulation were identified for integrating the biochemical data with the whole genome expression data. Most interestingly, the genes related to these metabolic pathways were found to be differentially expressed genes (DEGs) linked to floral transition (Table 1). Five genes related to chlorophyll metabolism including *ATPC1, atpH, CA, SECA1* and *LHCB1* encoding ATP synthase gamma chain, ATP synthase CF0 subunit IV, carbonic anhydrase, protein translocase subunit *SECA1* and chlorophyll A/B binding protein were found to be upregulated in the floral bud with the penultimate gene described in the table being non-significant. The chloroplastic ATP synthase gamma chain shared 97% homology with *Pistacio vera* and motif search could identify sucrose phosphate synthase and sucrose 6F-phosphate phosphohydrolase in vegetative and floral tissues sharing 100% homology with *Pistacio* and *Mangifera.* These results are in corroboration with the chlorophyll a and b content and total chlorophyll content in the biochemical analysis. Higher total chlorophyll content and enhancement in the chlorophyll a and b contents in the floral tissues also correlates to the expression of chlorophyll functional genes and these genes were located in the chloroplast. Further, total phenol content in the floral shoots was enhanced than in vegetative shoots and this could be related to the phenol catabolism catalyzed by polyphenol oxidase (PPO). PPO gene encoding polyphenol oxidase was also found to be significantly upregulated in the floral bud which could be attributed to the utilization of higher phenols and its oxidation.

Sugar metabolism is one of the important factors that are altered by stress which plays a significant role in the flowering process. The carbohydrate theory of

Table 1 — Identification of differentially regulated genes involved in biochemical pathways related to floral transition.

Pathway	Genes	Gene code	Function	Log2FC*	p-value	Significance/ regulation
Chlorophyll metabolism	ATP synthase gamma chain, chloroplastic	*ATPC1*	ATP Synthesis, Involved in photosynthetic electron transport system II	2.5384	0.0392	Significantly upregulated
	ATP synthase CF0 subunit IV, chloroplast	*atpH*	ATP Synthesis	3.6179	0.0203	Significantly upregulated
	Carbonic anhydrase, chloroplastic-like	*CA*	Carbonic anhydrase, chloroplastic-like	2.704	0.0250	Significantly upregulated
	Protein translocase subunit SECA1	*SECA1*	Central role in coupling the hydrolysis of ATP to the transfer of precursor proteins, serve as an ATP-driven molecular motor driving stepwise translocation of polypeptide chains across the membrane	3.4576	0.0326	Significantly upregulated
	Chlorophyll A/B binding protein	*LHCB1*	Functions as a light receptor, captures and delivers excitation energy to photosystems with which it is closely associated.	2.5387	0.17503	Non-significant upregulated
Phenol oxidation	Polyphenol oxidase, chloroplastic-like	*PPO*	Catalyzes the oxidation of mono- and o-diphenols to o-diquinones.	3.4380	0.0067	Significantly upregulated
Sugar metabolism and transport	Sucrose phosphate synthase	*SPS*	Synthesis of sucrose in photosynthetic and nonphotosynthetic tissues	4.9806	0.0014	Significantly upregulated
	Glucose-6-phosphate/phosphate translocator 2	*GPT2*	Required for dynamic acclimation of photosynthesis and partitioning of Glc6P between the chloroplast and cytosol.	4.4645	0.0006	Significantly upregulated
	Alpha, alpha-trehalose-phosphate synthase	*TPS1*	Required for vegetative growth and transition to flowering	-2.9641	0.0383	Significantly downregulated
	ABC transporter C family member 9-like isoform X1	*ABCC9*	Transmembrane transport	4.4318	0.0027	Significantly upregulated
	D-xylose-proton symporter-like 3, chloroplastic-like	*XYL*	Glucose membrane transport	3.7776	0.0074	Significantly upregulated
	Sugar phosphate translocator	*SPT*	Sugar translocator	0.7435	0.5219	Non-Significant upregulated
Anthocyanin metabolism	Chalcone synthases-1	*CHS1*	Involved in the pathway flavonoid biosynthesis	6.3173	9.8049	Significantly upregulated
	Naregenin-chalcone synthase family protein	*CHS*	Involved in the pathway flavonoid biosynthesis	-4.3656	0.0011	Significantly downregulated
	Leucoanthocyanidin dioxygenase-like isoform X1	*LDOX*	Involved in anthocyanin and protoanthocyanidin biosynthesis	-3.5467	0.0062	Significantly downregulated
	Anthocyanidin 3-O-glucoside 2"-O-glucosyltransferase-like	*3GGT*	Involved in the pathway anthocyanin biosynthesis, which is part of pigment biosynthesis	-2.8309	0.0229	Significantly downregulated
Auxin regulation	Auxin response factor	*ARF*	Transcription factor that bind to auxin response elements (AuxREs) in promoters of primary or early auxin responsive genes.	4.0182	0.0057	Significantly upregulated
	Auxin influx carrier protein	*AUX1*	Carrier protein involved in proton-driven auxin influx	6.2266	0.0001	Significantly upregulated
	ABC transporter G family member 19	*ABCG19*	Transmembrane transport of auxins	-5.2370	0.0014	Significantly downregulated
Gibberellic acid regulation	Gibberellin-regulated protein 4	*GASA*	Gibberellin-regulated protein involved in the regulation of floral meristem and floral organ identity	2.4586	0.0399	Significantly upregulated

Note: * Log2FC indicates logarithmic 2 fold change with respect to floral bud (FBD). Fold change (FC) values were initially determined based on base mean of floral bud (FBD vs base mean of vegetative bud (VBD). Negative values indicate down regulation and positive value indicates upregulation. Log2F $\geq$ 2.0 and P $\leq$ 0.01 were used as cut-off values for filtering out the significance level of gene expression. Based on these cut-off values, the regulation of differentially expressed genes have been described as significantly upregulated or downregulated with respect to FBD.

flowering also envisages the role of sugar metabolism to be one of the key master regulators of vegetative to floral transition[45]. Several genes encoding for sugar metabolism were detected with differential gene expression patterns like gene encoding alpha-trehalose phosphate synthase (significantly downregulated), sugar-phosphate synthase (significantly upregulated), transporter proteins (significantly upregulated) and sugar-phosphate translocator being non-significantly upregulated. Similarly, anthocyanin metabolism-related genes were mostly significantly down regulated except for *CHS1* encoding chalcone synthase.

Furthermore, significant upregulation of *GASA* gene encoding gibberellic acid regulated protein 4, was found that controls floral meristem formation and floral organ identity. Similarly, auxin accumulation was corroborated with the gene expression data by significant upregulation of *AUX1* and *ARF* genes and motif-related information that identified domains of *ARF* and *AUX* influx (Fig. 5). Significant down regulation of *ABCG9* also supports the movement of auxins to other tissues are curtailed and thus auxin accumulation in the floral bud is prioritized. Role of hormones was established in paclobutrazol induced flowering in another important cultivar Totapuri wherein GA3 and cytokinin contents were found to have opposite trends [46].

In conclusion, it can be postulated that increased active supply of sucrose synthase, sucrose phosphate synthase 1 to the flowering shoots plays an important role during swelling of buds, bud break and the emergence of panicle during flowering along with the establishment of auxin maxima. Regulation of GA3 well supported by up-regulation of chlorophyll metabolism affecting genes also play a central role in this transition (Fig. 6). Furthermore, transcriptomic datasets were utilized to identify genes associated with the studied parameters and corroborate the gene expression status based on p values. The increased accumulation of carbohydrate reserves and its metabolizing enzyme activities during the onset of flowering along with hormonal gradients were thereby

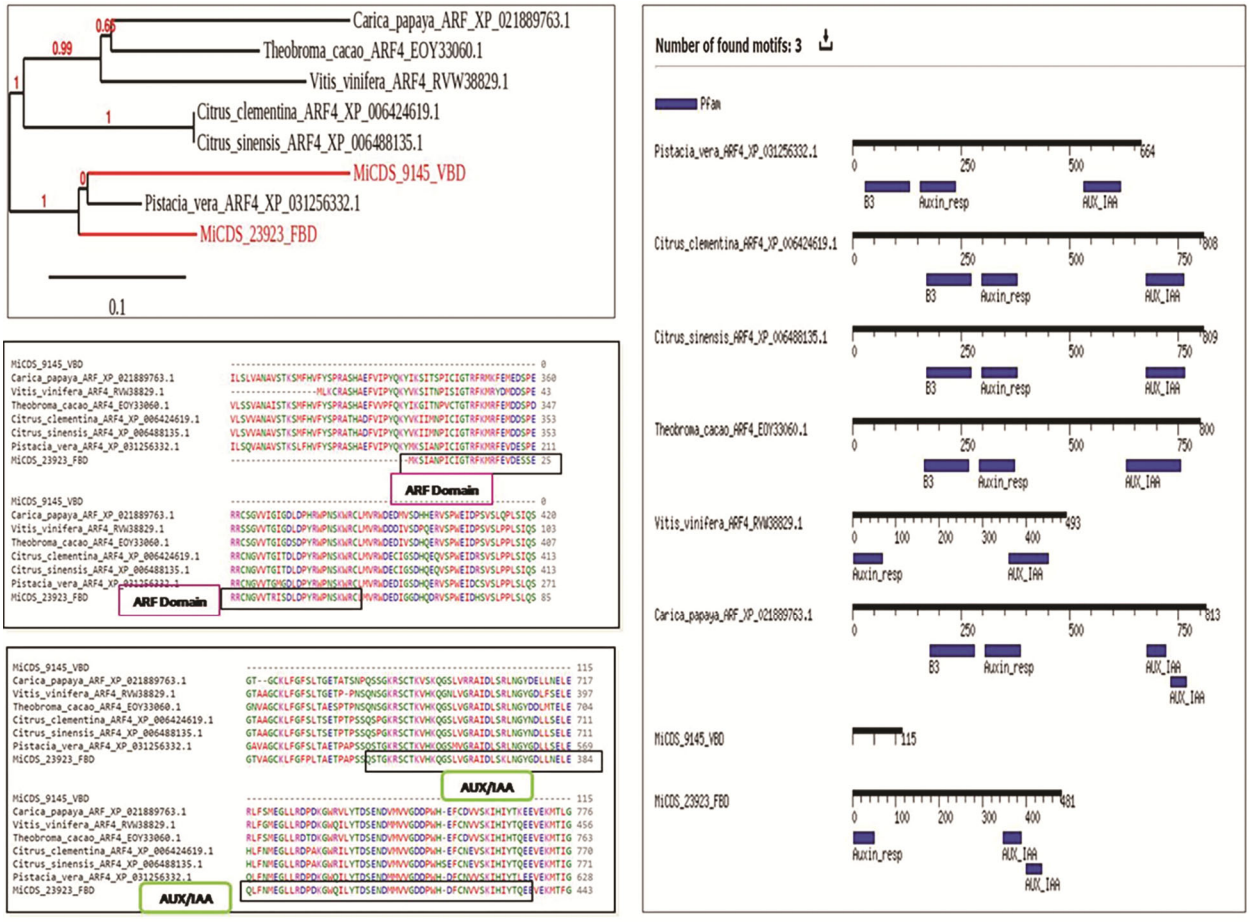

Fig. 5 — Phylogenetic analysis and motif identification of ARF & AUX/IAA domain in mango.

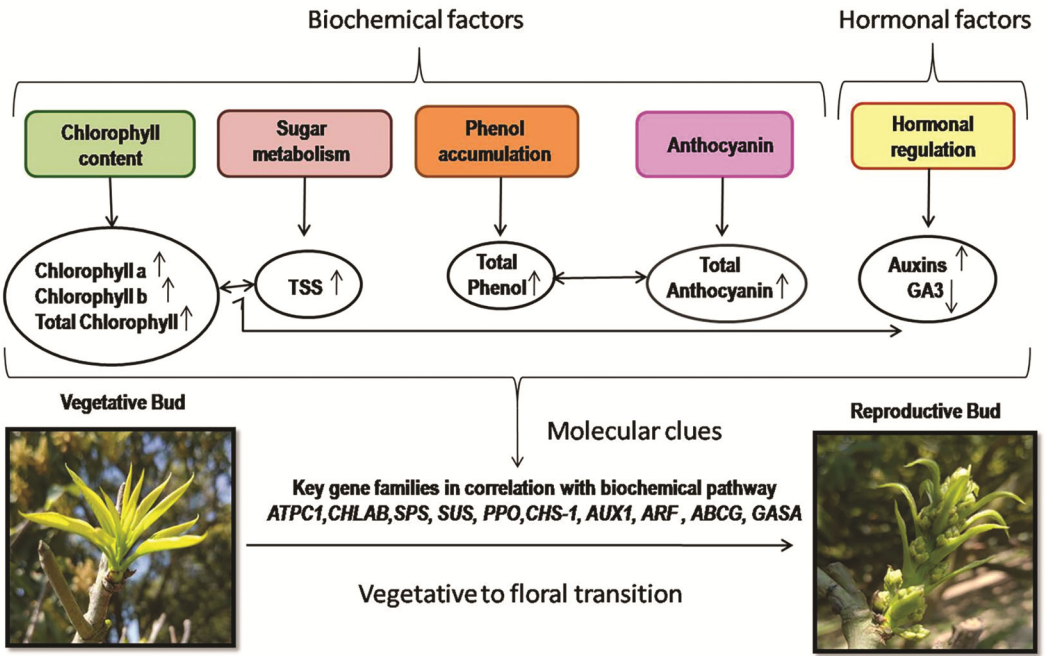

Fig. 6 — Floral transition in mango associated with upregulation of chlorophyll, sugar, phenol, flavonoids and auxin maxima establishment genes.

established to have a decisive role in the flowering processes in mango. Future prospects include real time q- PCR analysis by retrieving coding sequences related to identified DEGs from RNA sequencing data to further validate the *in silico* findings.

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